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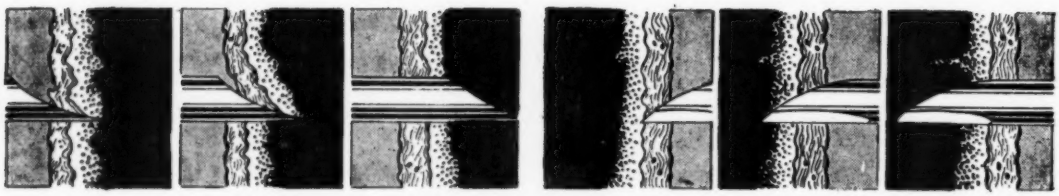

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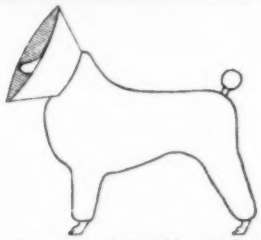

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An Outbreak of Infectious Synovitis in Chickens

BY

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SUMMARY.—An outbreak of disease in broiler chickens characterised by joint enlargement is described and a description is given of the experimental reproduction and treatment of the condition. The similarity of the disease to infectious synovitis as described by American workers is discussed.

Introduction

A NUMBER of different types of bacteria have been cited as the cause of arthritis or synovitis in poultry. Infection due to *Staphylococcus pyogenes* (Lucet, 1892) is probably most frequently seen but other bacteria have occasionally been implicated in arthritic diseases in chickens. *Bacterium arthropyogenes* (Nobrega, 1940) and *Escherichia venezuelensis* (Gallo, 1942) were isolated from swollen joints and each produced an arthritis when inoculated into chickens. Durant and McDougle (1932) recovered *Salmonella pullorum* from the joints of 2 chicks and outbreaks of disease in chickens, characterised by joint enlargement, have been found to be caused by variant strains of *S. pullorum* (Testi, 1958; Carnaghan & Sojka, 1958). *Salmonella typhimurium* has also been isolated from a case of arthritis in a mature fowl (Carnaghan, 1958). In laboratory experiments it has been found that certain serotypes of *Escherichia coli* produce an arthritis and synovitis when inoculated intravenously into chickens (Sojka & Carnaghan, 1958).

In addition to these bacterial causes of arthritis, pleuropneumonia-like organisms have been indicated as a cause of joint enlargement in fowls by Wills (1955) and Olson *et al.* (1956).

In 1954 in the United States, Olson *et al.* and Wills reported the discovery of an unidentified agent which produced a disease in chickens affecting primarily the synovial or bursal membranes. Wills (1955) suggested the designation Infectious Synovitis for the disease which he described as a subacute or chronic systemic disease of chickens characterised by purulent or caseous exudate in synovial tissues. There has been a rapid spread of this disease and it is now widespread in the broiler producing areas of the United States. Reporting on the disease in the Delmarva area, Cover *et al.* (1956) stated that morbidity was variable but had been as high as 30 per cent. while mortality was low, about 2 per cent. The economic importance of the disease could be measured primarily in the number of culls and crippled birds which it caused.

Although principally a disease of broiler chickens an outbreak of a similar condition in turkeys in the United States has been reported by Snoeyenbos and Olesiuk (1955) and turkeys are experimentally susceptible to the agent responsible for the disease in chickens (Wills, 1955, and Olson *et al.*, 1956). There appears to be general agreement among the American workers that the disease is caused by a large particle virus or viruses.

Apart from a suspected case of the disease in Canada, described by Bigland and Brown (1955), there are no reports of the conditions occurring outside the United States.

The purpose of this article is to describe an outbreak of disease in broilers in this country which was similar to the published descriptions of infectious synovitis and to report the experimental reproduction of the condition.

Field Outbreak

The birds in which the disease occurred consisted of 5,500 broilers in a house divided into two by wire

netting. Brooding losses had not been abnormally high, but at 6 weeks of age deaths had become numerous and a number of lame birds were seen in one half of the house and a week later birds in the other half of the house became affected. By the time they were 8½ weeks old 320 had died and it was estimated that at least another 10 per cent. were ailing. Twenty birds were examined *post mortem* and 2 distinct disease entities were found to be present. Several of the birds were found to have a fibrinous pericarditis, hepatitis and thickened air sacs containing caseous material. A pure growth of *Escherichia coli* was isolated from these cases and serological typing showed that the organism was a serotype which is commonly associated with mortality in broiler units and has been shown to be pathogenic in laboratory experiments (Sojka & Carnaghan, 1958).

The majority of the birds examined, however, showed no specific lesions in the viscera although in some of them there was enlargement of the liver and spleen. In all of them there was swelling of the joints, the hocks and feet being most frequently involved, but in some of them the wing joints were also affected. In the majority of these birds there was also swelling of the sternal bursa and the tendon sheaths between the hock and foot. The swellings were found to contain exudate varying from a grey viscous fluid to creamy yellow material or caseous exudate in advanced cases. Smears made from joint exudate and stained by Giemsa's stain were negative for bacteria. Cultures were made from the heart, liver, spleen and joints on to 5 per cent. sheep's blood agar plates, MacConkey's medium and into peptone broth. These were incubated at 37° C., aerobically and anaerobically, for 48 hours but did not show any significant bacterial growth.

On the basis of *post-mortem* examinations it was estimated that about two-thirds of the disease in the group was attributable to the synovial condition.

The whole group of birds was killed at 9 weeks of age and sent to a packing station whose returns showed that 30 per cent. of the birds were downgraded for poor carcase quality compared with an average figure of 8 per cent. for previous batches from the same premises.

Experimental Data

A quantity of pus was withdrawn aseptically from the hock joint of an affected bird and diluted 1 in 10 with sterile broth. Twenty thousand units of penicillin were added to each ml. of inoculum which was left at room temperature for 2 hours before use. Six 4-week-old chicks were inoculated into the foot-pad with 0.25 ml. of this inoculum and another 6 of the same age were inoculated with sterile broth to serve as negative controls. A further group of 6 chicks were inoculated with a Seitz EK filtrate of the inoculum. Twelve 6-day-old embryonated chicken eggs were inoculated into the yolk sac with 0.25 ml. of the unfiltered inoculum.

The control group and the group inoculated with the Seitz filtrate remained healthy throughout the period of the experiment. All the chicks inoculated with the unfiltered inoculum developed swellings at the site of inoculation 4 to 5 days later and within a few days the hock joints and sternal bursae became swollen. The wing joints became similarly affected 10 to 14 days after the onset of symptoms. Four of the chicks died during a 6 weeks observation period, deaths occurring 16, 19, 20 and 24 days after inoculation. Affected chicks became lame and tended to assume a sitting position. The comb and wattles became pale and the feathers ruffled (Plate I). They tended to huddle together cheeping continually. Although the birds ate and drank until shortly before death there was considerable loss of weight and those that died were emaciated.

All the embryonated eggs died within 11 days of inoculation and 9 serial passages have since been carried out. A total of 119 6- or 7-day-old embryonated eggs have been inoculated *via* the yolk sac and only 2, neither of which hatched, have survived to 21 days. Pooled yolk from 2 or 3 embryos in each passage were used as inoculum for the next passage until passage 8 when embryonic livers were used. The inoculum in each case contained 20,000 units of penicillin per ml. The results of egg passage are shown in Table I.

Six 6-day-old embryonated hens' eggs inoculated *via* the yolk sac with 0.25 ml. of yolk, from embryos in passage 1, diluted 1 in 10 in broth, died within 7 days of inoculation but when 40 mg. of streptomycin were incorporated in each ml. of this inoculum no deaths occurred in six 6-day-old embryos inoculated in the same way.

The disease was transmitted to 4- to 5-week-old chicks using embryonic liver or yolk sac as inoculum

TABLE I

SUMMARY OF MORTALITY IN 6- AND 7-DAY-OLD EMBRYONATED HENS' EGGS INOCULATED *via* YOLK SAC

Passage	Date	Number of eggs	Mortality per cent.	Number of dead embryos—days post inoculation												
				1	2	3	4	5	6	7	8	9	10	11	12	13
1	26.3	12	100	0	2	2	1	0	1	0	0	3	2	1	—	—
2	10.4	11	109	0	3	0	0	5	2	1	—	—	—	—	—	—
3	18.4	12	100	0	2	0	1	4	4	0	0	1	—	—	—	—
4	14.5	12	100	0	0	0	0	9	3	—	—	—	—	—	—	—
5	21.5	12	100	1	0	0	3	2	2	1	0	0	1	1	1	—
6	28.5	12	91·6	0	1	1	4	4	1	—	—	—	—	—	—	—
7	5.6	12	91·6	0	1	1	3	1	2	2	0	0	0	1	—	—
8	12.6	12	100	0	0	3	4	1	1	1	0	0	1	0	1	—
9	18.6	12	100	0	0	0	1	5	2	0	2	0	1	0	1	—
10	24.6	12	100	0	1	1	1	5	4	—	—	—	—	—	—	—

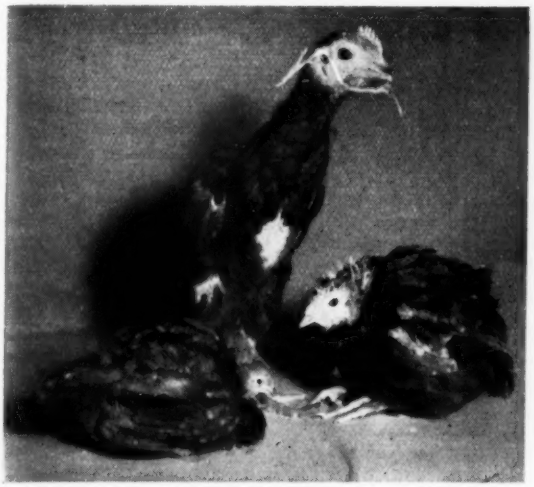

PLATE I.—Two experimentally infected chicks with healthy chick (centre).

from egg passage numbers 1, 3, 4, 7 and 10. Various routes of inoculation were used, *i.e.* intravenous, intramuscular, intrasinus and foot-pad, and all proved effective in reproducing the disease, when fresh material was used as inoculum. Joint exudate from the original field outbreak produced the disease in 3 out of 6 inoculated chicks after 45 days' storage in a refrigerator at 0° C.

The condition could not be reproduced by contact during a 3-week observation period and liver and joint exudate from a chick which died 35 days after experimental infection were non-effective when inoculated into the foot-pad of 6 chicks. A summary of chick inoculation is given in Table II.

TABLE II

SUMMARY OF CHICK INOCULATION EXPERIMENTS

Inoculation route	Inoculum	Number inoculated	Number affected	Remarks
Foot pad	Joint exudate ex field case	6	6	
Foot pad	Seitz filtrate of joint exudate	6	0	
Foot pad	Embryonic liver ex egg passage 1	6	6	
Foot pad	Joint exudate ex field case	6	3	After storage at 0° C. for 45 days
Foot pad	Yolk ex egg passage 3	6	6	
Foot pad	Yolk ex egg passage 4	4	4	
Intramuscular	Yolk ex egg passage 4	4	4	
Intrasinus	Yolk ex egg passage 4	4	2	
Foot pad	Yolk ex egg passage 7	6	6	
Contact	—	6	0	Duration of exposure 3 weeks
Intravenous	Yolk ex egg passage 10	6	6	

Three 3-week-old turkeys were inoculated into the foot-pad with 0.25 ml. of yolk from the sixth egg passage diluted 1 in 10 in broth. One of these developed synovitis although the disease was not as severe as in chicks, symptoms first being observed 12 days after inoculation, while another showed retarded growth and obvious illness but no synovial lesions. The third poult remained healthy. Three non-inoculated poults in the same accommodation did not develop the disease during a period of 4 weeks. Subsequently a further 3 young turkey poults were inoculated into the foot-pad with a 1 : 10 suspension of embryonic liver from egg passage 10. All developed synovitis but again the joint swellings were not as severe as in chicks and the incubation period of 9 days was longer than that in experimentally infected chicks.

Treatment

Twenty-four 3-week-old chicks were divided into 4 equal groups. The birds in 3 of the groups were inoculated into the foot-pad with 0.25 ml. of yolk *ex* egg passage 7 diluted 1 in 10 in sterile broth to which 20,000 units of penicillin per ml. of inoculum were added. The fourth group acted as a non-infected, non-medicated control. Group A were treated with chlortetracycline soluble* the dose being 600 mg. per gallon of drinking water (approximately equivalent to the chlortetracycline intake secured from 210 gm. per ton of food), treatment commencing at the time of infection. Group B were treated in the same manner but treatment was delayed until 5 days after inoculation when the whole group were visibly ill and swellings of the foot or hock joint were seen in 4 of them. Group C were not treated. The experiment last for 24 days and the results are summarised in Table III.

TABLE III

TREATMENT WITH CHLORTETRACYCLINE SOLUBLE

Group	Infected	Treatment	Affected	Died	Average weight at end of experiment
					Lb. oz.
A	Yes	Aureomycin from day of infection	0	0	1 1·8
B	Yes	Aureomycin 5 days after infection	4	0	1 1·5
C	Yes	Nil	6	4	7
D	No	Nil	0	0	1 1·8

Although the joint swellings in the chicks in Group B persisted throughout the period of the experiment there was no depression in growth rate and the birds appeared healthy. Twenty-four days after infection, medication was withdrawn from half of the chicks in Groups A and B and within a few days this had resulted in the development of synovitis in one of the chicks from Group A and in all 3 from Group B there was marked development of the joint swellings which had been quiescent

* Aureomycin.

during treatment. This suggests that in field outbreaks treatment should be continued until the birds are killed for the market.

Post-mortem Lesions

In birds killed in the early stages of the disease there was often marked enlargement of the liver and spleen. In a number of cases there was a green discoloration of the liver and this feature was particularly marked in one of the experimentally infected turkeys which became ill but did not develop synovial lesions. Birds dying from the disease were emaciated and severely dehydrated but there were seldom any visible lesions in the visceral organs. In early cases a clear viscous exudate was present in the affected joints and bursae. In some of the more advanced cases there were flakes of caseous yellow pus in the exudate or in others the exudate was creamy yellow. In long-standing cases the exudate tended to become caseous. The feet, hocks, together with flexor tendon sheaths between these joints and the sternal bursae were the most commonly affected sites, but in advanced cases lesions developed in the wing and mandibular joints.

PLATE II.—Swollen sternal bursa and right foot in experimentally infected chick.

PLATE III.—Swollen hock joints in experimentally infected chick.

Embryos dying 4 to 6 days after egg inoculation frequently had petechial haemorrhages on the head, eyelids, legs and back where they tended to coalesce but this feature was not seen in embryos dying later than this. In the majority of dead embryos the liver was enlarged, congested and contained irregularly shaped areas of yellow mottling. The spleen was enlarged and in some cases was between 6 and 8 times the normal size. There was marked congestion of the kidneys but the heart was ischaemic being almost white in some cases. In a few embryos there was a marked distension of the pericardium with a cloudy white fluid. The chorio-allantoic membrane was frequently oedematous and thickened. Joint lesions were not seen in the embryos.

Bacteriology

Repeated attempts to isolate bacteria from affected joints and from livers, hearts and spleens were negative. Material was sown into 5 per cent. sheep's blood agar, plain agar and MacConkey plates and into Selenite F and nutrient broth and incubated at 37° C., aerobically and anaerobically, for periods up to 10 days. On blood agar plates a faint zone of haemolysis occurred along the line of inoculation when culturing from the liver, but no colonies could be seen. No changes occurred in various sugar solutions nor did growth occur on artificial media for the isolation of pleuropneumonia-like organisms (P.P.L.O. enrichment broth, P.P.L.O. agar and Edward's P.P.L.O. agar).

PLATE IV.—Swollen right foot in experimental chick.

Discussion

The disease reported here appears to be similar, if not identical, to infectious synovitis as described in the United States. Although it was not possible to reproduce the disease with a Seitz EK filtrate the inability to grow any bacteria or pleuropneumonia-like organisms on artificial media suggests that the infective agent is probably a virus or viruses. Wills (1955) and Olson *et al.* (1956) reported inconsistent results in filtration experiments being unable to transmit the disease with Seitz EK filtrates although the former was successful using a Boerner centrifugal-type filter. Cover *et al.* (1956), in a study of 5 different strains of an agent causing synovitis, found that using Seitz filters, the filtrates from pads of 5.0μ and 2.0μ produced mortality in embryonated chicken eggs. Lecce *et al.* (1955) in electron micrograph studies described the agent as being 0.2 to 0.5μ in size and having properties most commensurate with rickettsia or possibly large viruses.

The agent isolated here, as judged by egg inoculation, was sensitive to streptomycin but resistant to penicillin. The majority of the American isolates of the synovitis agent have given similar results. In chick experiments chlortetracycline in the drinking water was shown to prevent the development of the disease when administered at the time of infection and to maintain normal growth when given 5 days after infection. Chlortetracycline has been found to be effective in the treatment of the experimentally induced disease in the United States by Shelton *et al.* (1957) who found that both chlortetracycline and oxytetracycline administered at the rate of 200 grammes per ton of food controlled the disease when fed continually but that oxytetracycline was less effective when treatment was delayed until joint swellings occurred. Withdrawal of treatment after 26 days medication, however, resulted in the reappearance of signs of synovitis in approximately 50 per cent. of treated birds (Bletner *et al.*, 1957). Furazolidone has also proved effective in field trials when given at a level of 100 to 200 grammes per ton of food (Cosgrove, 1957).

In the United States field outbreaks of infectious synovitis are usually associated with intercurrent flock disease, particularly the haemorrhagic syndrome and respiratory diseases (Olson *et al.*, 1957). In the disease outbreak reported here the condition was seen in association with an *Escherichia coli* infection in the flock which Gross (1956) regards as a complicating factor in chronic respiratory disease.

Other points of similarity with the American disease are the susceptibility of turkeys, the pattern of embryonic mortality in inoculated embryonated chicken eggs, the lesions in the embryos and the occurrence of haemolysis on blood agar plates when cultures were made from the liver.

The mode of transmission in naturally occurring outbreaks has not been finally established but Thayer *et al.* (1958) state that hatching egg transmission is very definitely indicated and this view is supported by Bankowski (1958) on the basis of his observations of field outbreaks of the disease. Transmission by contact was not accomplished here, possibly due to the limited contact period (*i.e.* 3 weeks) but has been successfully demonstrated by Wills (1955) and Olson *et al.* (1956). It would appear therefore, that under natural conditions the disease may originate as an egg-borne infection and may later spread by contact.

The confined conditions under which broilers are raised appears to render them particularly susceptible to this disease. Wills (1955) refers specifically to broilers in his report and Olson *et al.* (1957) found that all but one of the outbreaks he investigated occurred in broilers. Although only one outbreak of the disease has been seen here, infectious synovitis may well assume some economic importance to the developing broiler industry in this country if American experience of the disease is repeated here.

Acknowledgments.—The author wishes to express his gratitude to Mr. W. J. Sojka, M.R.C.V.S., for assistance with the bacteriological work, to Dr. W. M. McKay, M.R.C.V.S., who supplied the Aureomycin, to Mrs. D. Easthope and Miss H. Chilcott for technical assistance and to Mr. E. C. Clements for the photographs.

Addendum

Since this paper was submitted for publication, infectious synovitis has been diagnosed at Weybridge among broiler chickens on a further 8 premises.

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Chemotherapy of Caecal Coccidiosis in Chickens: The Activity of Nicarbazin

BY

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SUMMARY.—Nicarbazin at concentrations of 0·01 per cent. w/w or more in the food was effective in experimental caecal coccidiosis produced by serial doses or by a single dose of sporulated* Eimeria tenella *oöcysts, when medication was commenced before infection.*

At 0·025 per cent. w/w or more in the food it was effective in controlling caecal coccidiosis when treatment was delayed for 24 hours. If given 48 hours after infection, concentrations of 0·4 or 0·8 per cent. w/w showed only slight activity.

Nicarbazin did not prevent the development of immunity to caecal coccidiosis in chicks infected with E. tenella *oöcysts after medication had commenced.*

It affected the development of the second generation schizont but its action appeared to be predominantly coccidiocidal.

Introduction

CUCKLER, Malanga, Basso & O'Neill (1955), describing preliminary observations on molecular complexes of certain substituted carbanilides, stated that nicarbazin (an equimolecular complex of 4,4′-dinitrocarbanilide and 2-hydroxy-4,6-dimethylprimidine) effectively inhibited the development of *Eimeria acervulina*, *E. necatrix* and *E. tenella* in chicks. Cuckler, Malanga & Ott (1956) demonstrated that feeding 0·01 to 0·02 per cent. w/w nicarbazin prevented mortality from caecal and intestinal coccidiosis, but gave only partial protection when treatment was delayed for 48 hours after infection. Medication permitted the development of a high degree of immunity (Cuckler & Malanga, 1956).

Field trials supporting the efficacy of nicarbazin as a preventive against coccidiosis in chickens have been reported by Fogg & Siegmund (1956) and Fogg, Cuckler, Ott & Siegmund (1956) who recommended 0·0125 per cent. w/w of drug in the food as the optimum concentration.

Other investigators have confirmed the anticoccidial activity of nicarbazin (Barber, 1955; Rubin, McLoughlin, Costello & Wehr, 1956; Shumard & Eveleth, 1956; Marthedal & Velling, 1957).

In view of these findings it was thought desirable to undertake some confirmatory work in order to establish an experimental basis for the use of this drug in the United Kingdom.

Methods

Day-old chicks were obtained from a single commercial hatchery and isolated as far as possible from the risk of accidental infection. Rhode Island Red × Light Sussex chicks were used in most of the experiments, but occasionally Light Sussex × Rhode Island Red and Black Leghorn × Light Sussex chicks were employed.

Twenty-four hours before being placed on test, the chicks, in all but 2 experiments, were transferred to wire-floored metal cages in an experimental room which was maintained at a temperature of approximately 90° F. The light schedule was automatically regulated to give a 19-hour light period alternating with a 5-hour dark period. The chicks in Experiment 1 of Table I and Experiment 4 of Table II were housed in wire-floored wooden cages with continuous lighting.

TABLE I

COMPARISON OF THE EFFECTS OF NICARBAZIN, NITROFURAZONE AND SULPHAQUINOXALINE IN CAECAL COCCIDIOSIS OF YOUNG CHICKS

Experiment number	Compound	Percentage in food	*Deaths from caecal coccidiosis	*Mean weight (g.) gain per survivor
1	Nil (uninfected control)	—	0/26†	105·5
	Nil (infected control)...	—	13/26	47
	Nicarbazin	0·0125	3/26	101
	Nitrofurazone ...	0·011	4/26	92
		0·0055	9/26	78
	Sulphaquinoxaline ...	0·0125	9/26	60·5
2	Nil (uninfected control)	—	0/20	64·5
	Nil (infected control)...	—	13/20	44
	Nicarbazin	0·0125	1/20	54
	Nitrofurazone ...	0·011	1/20	57
	Sulphaquinoxaline ...	0·0125	4/20	36
3	Nil (uninfected control)	—	0/20	55
	Nil (infected control)...	—	19/20	—
	Nicarbazin	0·0125	3/20	53·5
	Nitrofurazone ...	0·011	6/20	30
	Sulphaquinoxaline ...	0·025	7/20	35
		0·0125	14/20	14·5
4	Nil (uninfected control)	—	0/20	54·5
	Nil (infected control)...	—	19/20	—
	Nicarbazin	0·0125	3/20	39·5
	Nitrofurazone ...	0·011	4/20	37
		0·0055	12/20	33
	Sulphaquinoxaline ...	0·025	6/20	26·5
		0·0125	11/20	19

* In Experiment 1 results recorded on the 8th day after infection; chicks 8 days old at beginning of test; medication commenced 3 days before infection. In Experiments 2, 3 and 4 results recorded on the 7th day after infection; chicks 6 days old at beginning of test; medication commenced 2 days before infection.

Numerator = number dead.

† Denominator = number of chicks used.

* Marketed in the U.K. under the brand names "Nicoxin" and "Nicrazin."

TABLE II

EFFECTS OF NICARBAZIN AND SULPHAQUINOXALINE ON THE DEVELOPMENT OF IMMUNITY TO *Eimeria tenella* WHEN FED DURING A PRIMARY INFECTION

Experiment number	Primary infection				† Challenge infection (200,000 oöcysts)		
	Oöcyst dose	* Compound	Percentage in food	Mortality by 7th day	Number chicks receiving challenge	Mortality by 7th day	Number chicks showing no caecal lesions
1	100,000	Nil (control)	—	8/10	—	—	—
		Nicarbazin	0·02	1/10	9	0	9
			0·015	1/10	9	0	9
			0·01	4/10	6	0	2
	Nil	Nil	—	0/10	10 (control)	6	0
2	100,000	Nil (control)	—	17/20	—	—	—
		Nicarbazin	0·0125	2/19	15	0	12
	5,000	Nil (control)	—	1/10	9	1	7
		Nicarbazin	0·0125	0/10	10	0	1
	Nil	Nil	—	0/15	15 (control)	6	0
3	2,000	Nil (control)	—	6/19	13	0	8
		Nicarbazin	0·0125	0/10	10	0	1
		Sulphaquinoxaline	0·025	0/20	20	0	4
			0·0125	2/20	18	1	8
	Nil	Nil	—	0/20	20 (control)	12	0
4	1,000	Nil (control)	—	2/22	20	1	9
		Nicarbazin	0·02	0/22	20	0	0
			0·01	0/22	20	1	1
			0·005	0/22	20	0	3
	Nil	Nicarbazin	0·1	0/10	10	5	0
			0·02	0/10	10	6	0
	Nil	Nil	—	0/22	20 (control)	8	0

* Medication given for 8 days commencing 24 hours before infection in Experiments 1 and 2, and for 9 days commencing 48 hours before infection in Experiments 3 and 4.

† Challenge dose given 10 days in Experiment 2, 7 days in Experiments 1 and 3, and 3 days in Experiment 4 after the last death from the primary infection in control group, *i.e.* after the 7th day.

the heat being supplied by dull emitter infra-red lamps. The chicks had access to food and water at all times.

Preparation of Medicated Food. In 3 experiments pure nicarbazin was employed after being ground in with plain wheat flour to form a smooth 10 per cent. w/w premix. Commercial nicarbazin premix, usually diluted to a concentration of 2·5 per cent. w/w in chick mash, was used for preparing the ration in all other tests. This premix contained nicarbazin in corn distillers' dried grains, wheat standard middlings and dried extracted vitamin B_{12} fermentation solubles. Up to 2·5 kg. of medicated food was prepared by thoroughly mixing the premix with a proprietary baby chick mash for at least 10 minutes in a " Kenwood Major " electric mixer, and quantities of food up to 7·0 kg. were mixed for at least 20 minutes in a " Hobart " mixer. Nitrofurazone and sulphaquinoxaline were incorporated in the food in the same way using the manufacturer's premixes (1·1 per cent. w/w " Nefco " ; 22·5 per cent. w/w " Embazin " premix).

Organism and Infections. The strain of *Eimeria tenella* was kindly supplied by Dr. C. Horton-Smith, of the Houghton Poultry Research Station, and has since undergone fairly regular passage through 6- to 10-day-old chicks. For the most part, the conventional method for collecting oöcysts from the faeces of infected chicks was followed, but occasionally oöcysts were obtained from the caeca of chicks 8 days after inoculation with sublethal doses of sporulated oöcysts as described by Waletzky & Hughes (1946).

Chicks were infected orally, and except where otherwise stated, each chick received approximately 200,000 sporulated oöcysts.

Oöcyst Counts. In one experiment, the entire daily output of faeces of each group of chicks was collected and used for the estimation of the total numbers of oöcysts passed. These were determined by the McMaster technique.

Histological Examination. Caeca were taken from treated and untreated chicks infected with approximately 100,000 sporulated oöcysts of *E. tenella.* The tissues were fixed in Carnoy's fixative or 10 per cent. formol saline, and sections cut at a thickness of 6 microns were stained with aqueous iron haematoxylin following the method of Heidenhain, with haematoxylin-eosin or with Giemsa's stain (Shortt & Cooper, 1948). The dimensions of the schizonts in the sections, which were cut from near the distal portion of the caecum in each case, were measured by means of a calibrated microscope eye-piece. Scrapings of the caecal wall stained with Giemsa's stain were also examined.

Results

The Effect of Medication Started before Infection Produced by a Single Dose of Sporulated Oöcysts

In the first preliminary experiments various concentrations of nicarbazin were examined for activity by our standard prophylactic procedure. Seven-day-old Rhode Island Red × Light Sussex pullets in groups of 10 to 20 were balanced for weight and

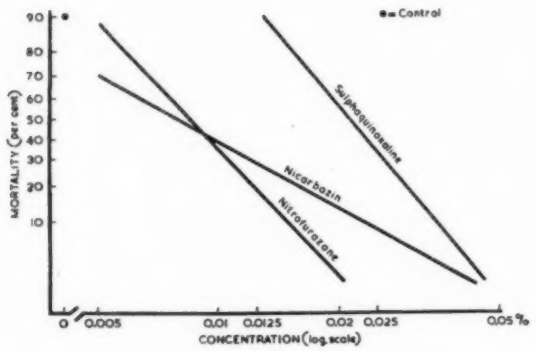

FIG. 1.—Dose-response curves showing the relationship between the concentration of nicarbazin, nitrofurazone and sulphaquinoxaline in the food and mortality in caecal coccidiosis when medication commenced before infection.

given drug-diets 24 hours before infection. The results of 20 replicate tests using approximately 1,000 chicks are given as a dose-response curve in Fig. 1. Results with nitrofurazone and sulphaquinoxaline have been included for comparison. Nicarbazin and nitrofurazone had approximately equal effectiveness at the concentrations recommended for continuous medication (*i.e.* 0·0125 and 0·011 per cent. respectively), and the same activity was shown by sulphaquinoxaline at approximately 0·025 per cent. In all tests the inoculum produced a consistently high mortality in the untreated control groups, and the mortality in the 12 tests using 0·0125 per cent. nicarbazin ranged from 0 to 40 per cent. Deaths from acute caecal coccidiosis in the nicarbazin treated groups occurred on the same days as deaths in the untreated control groups, and in all infected groups fed nicarbazin blood was passed in the droppings on the 5th and 6th days after infection. No delayed bleeding or deaths occurred up to 7 days after withdrawal of medicated food. In 3 of these experiments the caecal lesions were classified at the time of death or in the survivors on the 7th day after infection, and very severe lesions were present in 25 out of 30 untreated infected controls. Four out of 20 chicks given 0·0125 per cent. nicarbazin showed very severe lesions, but no lesions of this severity were recorded from 10 chicks fed 0·025 per cent. drug.

In 3 other experiments using 20 chicks per group, 0·0125 per cent. w/w nicarbazin in the food prepared from a 10 per cent. premix of pure drug in wheat flour, showed activity similar to that shown in Fig. 1 at that concentration.

Nicarbazin at 0·0125 per cent. w/w was fed to one group of 30 Rhode Island Red × Light Sussex pullets for 7 days prior to infection and to another group of 27 chicks for one day before inoculation which took place when the chicks were 8 days old. Three of the 30 chicks in the first group and 4 of the 27 in the second group died of acute caecal coccidiosis in contrast with 25 deaths from an infected untreated control group of 29 birds. This confirmed the preliminary findings and indicated that feeding nicarbazin for 24 hours prior to infection was sufficient time to permit a good assessment of activity under experimental conditions.

Mash containing nicarbazin was fed for 8 days to 4-week-old Rhode Island Red × Light Sussex pullets commencing 24 hours before infection. Fourteen birds were used in each group in this single experiment. Seven of the untreated infected birds and 6 of those receiving 0·01 per cent. nicarbazin succumbed, whereas all the birds fed 0·02 per cent. of the drug survived.

A Comparison of the Effects of Nicarbazin, Nitrofurazone and Sulphaquinoxaline. In 4 experiments, recorded in Table I, the efficacy of nicarbazin, nitrofurazone and sulphaquinoxaline was compared when given to young Rhode Island Red × Light Sussex chicks for 9 or 11 days starting before inoculation which produced 2 moderate and 2 severe infections. Pullets were used in Experiments 1 and 3, and cockerels in Experiments 2 and 4. Blood was passed in the faeces on the 5th, 6th and often the 7th day following the ingestion of oöcysts in the nicarbazin and sulphaquinoxaline treated groups and in the infected controls. The majority of deaths in the nicarbazin groups occurred on the 5th day after infection, whereas in nearly every case deaths and the passage of blood in the groups of chicks fed nitrofurazone were delayed until the 6th day. The chicks fed nicarbazin and nitrofurazone at about the same concentrations showed little difference in mortality and weight gains, but under these conditions 0·0125 per cent. sulphaquinoxaline was less effective.

The Effects of Nicarbazin and Sulphaquinoxaline on the Development of Acquired Immunity. The effects of medication on the development of immunity by 1-week-old chicks (Rhode Island Red × Light Sussex pullets in Experiments 1 and 2 ; Black Leghorn × Light Sussex cockerels in Experiment 3 ; Light Sussex × Rhode Island Red pullets in Experiment 4) exposed to various degrees of infection are shown in Table II. The chicks were infected with primary oöcyst doses 1 or 2 days after starting medication which was continued until the 7th day after this infection when the chicks were returned to normal ration. In each experiment one untreated group was given the initial infection and another group, reared under the same conditions, remained untreated and uninfected until the "challenge" inoculation, administered 3, 7 or 10 days after the withdrawal of medication. On the 8th day after the " challenge " infection the surviving birds were killed and examined for caecal cores and macroscopic caecal lesions. Against the severe primary infections (Experiments 1 and 2) nicarbazin at 0·0125, 0·015 and 0·02 per cent. w/w gave good protection and none of the survivors of these groups succumbed to the moderate infection produced by the " challenge " oöcyst dose and only a few chicks showed caecal lesions. Small infective doses of sporulated oöcysts, sufficient to produce a high degree of immunity in the untreated control chicks, were used in Experiments 2, 3 and 4. The results indicate that nicarbazin and sulphaquinoxaline at the concentrations used allowed a firm immunity to be established. The deaths in the two previously

TABLE III

COMPARISON OF THE EFFECTS OF CONTINUOUS MEDICATION WITH NICARBAZIN AND SULPHAQUINOXALINE ON THE DEVELOPMENT OF IMMUNITY TO *Eimeria tenella*

Medication	Primary infection: Percentage in food	Primary infection: Oöcyst dose	Primary infection: Deaths by 6th day	Deaths from challenge infection (100,000 oöcysts)
Nil (control) ...	—	800	0/19	2/19
		4,000	4/20	0/16
		8,000	6/20	0/14
Nicarbazin ...	0·0125	800	0/20	0/20
		4,000	0/20	0/20
		8,000	0/20	0/20
Sulphaquinoxaline	0·0125	800	0/20	5/20
		4,000	1/19	1/18
		8,000	2/20	1/18

Control : untreated and uninoculated till challenge 18/20

uninfected but medicated groups of Experiment 4 showed that there was insufficient drug remaining to be effective against the " challenge " infection.

The test recorded in Table III was carried out to determine the effect of treatment with 0·0125 per cent. w/w nicarbazin and 0·0125 per cent. w/w sulphaquinoxaline upon the acquisition of immunity when medication started 48 hours before the initial infection and continued throughout the test. The " challenge " inoculum was given 10 days after the primary infection. Under these circumstances fewer deaths occurred from severe infections than in previous experiments (Fig. 1 and Table I).

The Effect of Nicarbazin when a Course of Medication Started before Infection produced by Serial Doses of Sporulated Oöcysts

These experiments were undertaken to determine the effectiveness of nicarbazin on repeated infections during medication of Rhode Island Red × Light Sussex cockerels. The results given in Table IV show that a marked protection was provided by 0·01 and 0·02 per cent. w/w nicarbazin against moderate infections produced by this technique.

The survivors from the 3 medicated groups infected by serial doses of 5,000 sporulated oöcysts given 3 times a day for 5 days were taken off drug on the 7th day after the first oöcyst dose. Fourteen days later, these chicks, together with the 5 survivors of the infected untreated controls and 6 hitherto uninfected unmedicated chicks of the same age, were each inoculated with approximately 400,000 sporulated *E. tenella* oöcysts. None of the previously infected chicks died, but the " challenge " dose killed 5 of the 6 controls. This indicates that a firm immunity to the disease had been developed in the treated birds.

TABLE IV

EFFECT OF NICARBAZIN ON INFECTIONS PRODUCED BY SERIAL DOSING OF OÖCYSTS WHEN MEDICATION WAS COMMENCED BEFORE THE FIRST DOSE OF OÖCYSTS WAS ADMINISTERED

Percentage nicarbazin in food	Mortality by 7th day after first oöcyst dose: *5,000 oöcysts thrice daily for 5 days	†50,000 oöcysts twice daily for 2 days	*64,000 oöcysts daily on 3 alternate days
Nil (control)	7/12	6/10	5/8
0·02 ...	0/11	1/10	—
0·01 ...	0/12	2/10	1/9
0·005 ...	6/12	—	—

* Medication commenced 3 days before the first dose of oöcysts was administered.

† Medication commenced 1 day before the first dose of oöcysts was administered.

The Effect of Nicarbazin when Treatment was Started after Infection

Groups of Rhode Island Red × Light Sussex pullets were given treatments starting at 24 to 72 hours after infection and in every case continuing until the 7th day following the inoculation. The results, given in Table V, show that when medication commenced 24 hours after infection 0·05 per cent. w/w nicarbazin prevented all deaths, but diets containing up to 0·8 per cent. w/w of drug had little anticoccidial action when the start of treatment was delayed for 48 hours or more. There was a considerable weight gain depression and reduction in food intake by the chicks given 0·8 per cent. nicarbazin in Experiment 2, compared with the infected controls.

TABLE V

EFFECTS OF NICARBAZIN, NITROFURAZONE AND SULPHAQUINOXALINE ON THE SURVIVAL OF 7-DAY-OLD CHICKS WHEN TREATMENTS STARTED AFTER INFECTION WITH *Eimeria tenella* OÖCYSTS

Experiment number	Compound	Percentage in food	Time medication started after infection (in hours)	Deaths by 7th day after infection
1	Nil (control) ...	—	—	9/10
	Nicarbazin ...	0·025	24	2/10
			48	7/10
		0·05	24	0/10
			48	10/10
		0·1	48	8/8
		0·2	48	10/10
	Nitrofurazone ...	0·022	48	2/9
	Sulphaquinoxaline	0·05	48	3/10
2	Nil (control) ...	—	—	10/10
	Nicarbazin ...	0·4	48	5/10
			72	10/10
		0·8	48	8/10
			72	8/10
	Nitrofurazone ...	0·022	48	0/10
	Sulphaquinoxaline	0·05	48	0/10

The Effect of Nicarbazin Medication on the Development of E. tenella

The effect of 0·0125 and 0·025 per cent. w/w nicarbazin in the food upon the development of the coccidium was studied by examining sections of the caecal walls of chicks infected 24 or 48 hours after the commencement of treatment. These observations are summarised in Table VI. There was little difference in the size of the second generation schizonts in the treated and untreated chicks at the 5th and 6th days after infection, but the number of schizonts seen in

TABLE VI

THE EFFECT OF NICARBAZIN MEDICATION ON THE DEVELOPMENT OF *E. tenella*

Experiment number	Medication	Time (hours) after infection that caecal sections were taken		
		96	120	144
1	Nil (control)	Early 2nd generation schizonts : 13·5 × 9·8 microns. Few red blood cells in lumen of caecum	Advanced 2nd generation schizonts : 24 × 17 microns. Profuse haemorrhage	2nd generation schizonts : 28 × 17 microns. Micro- and macrogametocytes
	0·0125 per cent. nicarbazin in the food started 24 hours before infection	2nd generation schizonts : 9 × 6·7 microns	Advanced 2nd generation schizonts : 30·8 × 19·6 microns. Few red blood cells in lumen of caecum	2nd generation schizonts : 27 × 19 microns. Few gametocytes
2	Nil (control)	Early 2nd generation schizonts : 12·8 × 9 microns. Slight haemorrhage	Advanced 2nd generation schizonts with merozoite formation : 26 × 18·4 microns. Profuse haemorrhage	2nd generation schizonts : 33·4 × 21 microns. Micro- and macrogametocytes.
	0·0125 per cent. nicarbazin in the food started 48 hours before infection	Few early 2nd generation schizonts : 6·6 × 5·6 microns	2nd generation schizonts : 25·1 × 17·6 microns, a few with merozoite formation. Slight haemorrhage	Few 2nd generation schizonts : 34·8 × 24·9 microns. An occasional gametocyte. Blood in lumen of caecum
	0·025 per cent. nicarbazin in the food started 48 hours before infection	—	Few 2nd generation schizonts : 26·3 × 16·7 microns. Very occasional merozoite formation. Slight haemorrhage	Few 2nd generation schizonts: 32·1 × 21 microns. Macrogametocytes. Slight haemorrhage

the sections from treated chicks was much less than that in the controls. The contents of some of the schizonts in the treated birds appeared abnormal. The rate of development of the parasite seemed only slightly retarded by the medication, and the action of nicarbazin appeared to be mainly coccidiocidal at the two concentrations studied. An examination of scrapings of the caecal mucosa substantiated these findings.

The Effect of Nicarbazin Medication on Oöcyst Output

Nicarbazin at 0·006, 0·0125 and 0·025 per cent. w/w was offered to 3 groups of 7-day-old Rhode Island Red × Light Sussex cockerels 24 hours before each chick received approximately 4,000 sporulated oöcysts of *E. tenella*, and medication was continued for 14 days. An untreated infected group was used for comparison and an untreated uninfected group was kept to serve as a control against the possible entry of extraneous infections. No oöcysts were seen in the faeces of this latter group throughout the test. Each group contained 10 chicks and no deaths occurred during the experiment. The results are illustrated in Fig. 2. Nicarbazin fed at 0·0125 and 0·025 per cent. decreased oöcyst output, and the time of oöcyst production from the chicks given the highest drug concentration indicated a retardation in the rate of development of the parasites as well as destruction of some of the stages.

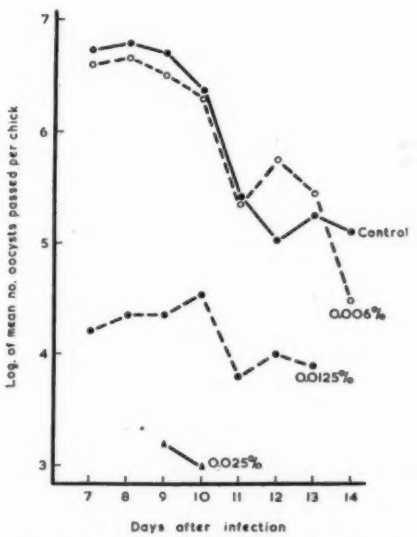

FIG. 2.—Effect of nicarbazin (0·006, 0·0125 and 0·025 per cent.) on the number of oöcysts passed in the faeces by infected chicks. (On the 14th day after infection no oöcysts were seen in the faeces of the group fed 0·0125 per cent.).

Discussion

In general these results confirmed those obtained by American workers, although for the most part our infections were more severe.

Nicarbazin mixed in the food at concentrations of 0·01 to 0·05 per cent. w/w when given to young chicks before the administration of sporulated *E. tenella* oöcysts, prevented or reduced the mortality from experimental caecal coccidiosis. However, chicks receiving these concentrations of drug 48

hours after infection were not saved. The effects of treatment with 0·0125 per cent. w/w nicarbazin (the recommended concentration for continuous medication) starting before infection and continuing for 7 or 8 days after, were compared with those of 0·011 per cent. w/w nitrofurazone and 0·0125 per cent. w/w sulphaquinoxaline all incorporated in the food. The results showed sulphaquinoxaline to be inferior to the other two compounds which had approximately equal activity at the concentrations examined.

There was no evidence that nicarbazin treatment of chicks exposed to infections of *E. tenella* prevented or delayed the development of their immunity to this species (Table II), although an examination of the caecal lesions produced by the " challenge " inoculations suggested that birds given small doses of oöcysts when being fed nicarbazin were subsequently more severely affected than the survivors of the untreated infected groups.

The mortality in groups of chicks fed 0·0125 per cent. w/w nicarbazin commencing before the inoculation of sufficient oöcysts to produce a severe infection was usually not less than 10 per cent. (Table I and Fig. 1). On the other hand, when a small dose of oöcysts preceded the heavy inoculum, which killed 90 per cent. of the untreated controls, no deaths occurred (Table III), probably due to the development of immunity in the presence of drug following the initial infection.

The mode of action of nicarbazin is not fully known. Basso & O'Neill (1954) and Ott, Kuna, Porter, Cuckler & Fogg (1956) showed that the anticoccidial activity of the physical mixture of the two components of nicarbazin was no greater than the activity of 4,4′-dinitrocarbanilide alone, which was significantly less than the complex. Tests in these laboratories have confirmed this and also showed that nicarbazin did not give a greater response when given in conjunction with sulphaquinoxaline or pyrimethamine, and its activity was not reversed by equimolar amounts of para-aminobenzoic acid.

Nicarbazin at 0·0125 and 0·025 per cent. w/w caused a delay in reaching the peak of oöcyst production and smaller numbers of oöcysts were produced from these treated chicks in comparison with the untreated control group. A study of sections and scrapings of the caecal walls from treated and untreated infected chicks showed that the number of second generation schizonts was greatly reduced by nicarbazin medication and some of those present in treated chicks showed abnormal and slightly retarded development. Nicarbazin probably destroys some stages and inhibits the development of others, but these findings were from 2 experiments only and it was not established whether earlier developmental stages of the parasite were affected.

No toxic symptoms were noticed in chicks fed low concentrations of nicarbazin for short periods in our tests, and it has been found that continuous feeding of 0·0125 per cent. w/w nicarbazin to chickens up to 11 or 12 weeks of age does not depress weight gains (Cuckler, Malanga & Ott, 1956 ; Fogg *et al.*, 1956 ; Newberne & Buck, 1957 ; Lucas, 1958). Some temporary adverse effects on egg-shell colour, the degree of mottling of egg yolks, hatchability and egg production have been reported by Ott, Dickinson & Peterson (1956), Ott, Kuna, *et al.* (1956), Polin & Porter (1956), Sherwood, Milby & Higgins (1956), Sherwood, Milby and Witz (1956), Baker, Hill, van Tienhoven & Bruckner (1957), McLoughlin, Wehr & Rubin (1957), Weiss (1957), and Lucas (1958). Consequently the drug is contra-indicated for laying hens.

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SUPPLEMENT TO "THE VETERINARY RECORD"

The next Supplement is now in course of preparation and honorary secretaries of Divisions and others likely to have manuscript for publication would assist the Editor if they would be good enough to forward material not later than the end of the third week in February.

Meningo-encephalitis Eosinophilica of Swine

II. Studies on the Experimental Reproduction of the Lesions by Feeding Sodium Chloride and Urea

BY

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SUMMARY.—Pigs fed on diets containing 5·3 and 9·4 per cent. of sodium chloride, with water restricted to about 2½ times the weight of dry food, developed typical signs and lesions of meningo-encephalitis eosinophilica.

The syndrome was also produced by feeding 3 per cent. sodium chloride + 7·5 per cent. urea with restricted water, but when a further 7·5 per cent. urea was substituted for the sodium chloride (giving a total of 15 per cent. urea), perivascular eosinophile infiltration of the cerebrum did not appear though the histopathological picture was otherwise similar. The feeding of 3 per cent. sodium chloride with the same allowance of water produced no significant clinical or histological abnormality.

It is suggested that cerebral midcortical laminar malacia with vascular endothelial proliferation and perivascular lymphocytic infiltration may result from disturbed water balance arising from various causes, but that the pronounced eosinophile infiltration in meningo-encephalitis eosinophilica is a characteristic effect of the sodium ion.

It is recommended that sodium chloride should not be added to the compounded diets of pigs and that unrestricted water should always be available.

Introduction

IT is now well recognised that meningo-encephalitis eosinophilica of swine is closely associated with poisoning by sodium salts while on a restricted water intake (Smith, 1955 ; Done, Brooksbank & Buntain, 1957 ; Hjärre & Öbel, 1956 ; Smith, 1957). The authors' interest in the role of sodium chloride in the pathogenesis of the disease was aroused by the outbreaks described by Done, Brooksbank & Buntain (*op. cit.*). Following the experimental reproduction of the condition with sodium chloride, some attempt was made to investigate the specificity of this agent for the production of the various lesions making up the complete picture. The literature contains many references to the typical signs and lesions of meningo-encephalitis eosinophilica (Michalka, 1941 ; Larsen, Moller & Thordal-Christensen, 1955 ; Smith, 1957) so only a brief account is given of the early experiments using sodium chloride alone. The later proceedings are given in more detail.

Material and Methods

Most of the pigs used in the experiments were Large Whites or Large White crosses which were purchased from commercial sources. Those used in Experiment IIIc were the progeny of a sow kept at the laboratory, who had had contact with Talfan disease with her preceding litter. The pigs used in Experiment I had been vaccinated against swine fever with crystal violet vaccine and had survived challenge with virulent virus 3 weeks previously. The sodium chloride used in Experiment I was a vacuum-dried salt obtained from the same source as a batch of meal which had caused poisoning in the field. Analysis of this product did not reveal significant quantities of salts other than sodium chloride. Analytical grade sodium chloride and urea were used in the other experiments. In order to provide competition as an inducement to consume the rather unpalatable feed, trough space inadequate to the number of pigs was provided; initially 2 lb. of meal per pig were given daily, but in view of the animals' reluctance to eat their full allowance they were later fed to appetite at each meal and the total weight of food consumed was calculated at the end of the experiment. Figures of total salt consumed per pig each day assume that appetite was the same in each pig and that the amount eaten varied as the number of feeds given, and are probably very approximate.

Pigs were killed by the intracardiac injection of pentobarbitone sodium, followed by exsanguination, and a sample of cardiac blood was usually taken immediately before this operation. Urine samples were taken at necropsy. Serum chloride was estimated by the method of Whitehorn (Hawk, Oser & Summerson, 1949). For histopathological examination of the central nervous system, standard blocks were taken as follows :—

(1) One or more transverse sections of one or both cerebral hemispheres.

(2) Transverse section through the anterior corpora quadrigemina.

(3) Longitudinal section through the cerebellar vermis.

(4) Transverse section through the medulla oblongata at the level of the obex.

(5) Transverse section through the lumbar spinal cord.

Tissues were fixed in 10 per cent. formol saline, embedded in paraffin, sectioned at 5 to 7 microns, and stained by Ehrlich's haematoxylin and eosin and in some cases by Masson's trichrome stain.

Experiment I

Vacuum dried salt was thoroughly mixed with a proprietary fattening meal to give a final concentration

of 5·3 per cent. by weight. A 2-gallon bucket was half filled with meal and water added till the bucket was nearly full.

Feeding of the experimental mixture to 7 pigs commenced on the evening of 19.7.55. Slight abnormalities of behaviour were evident in P.1397 on 22.7.55, and in the remaining pigs on 23.7.55.

On 24.7.55, P.1397 began to show typical signs of meningo-encephalitis eosinophilica. The remaining pigs were dull and showed some intention tremor.

Owing to an unforeseen change of attendant the pigs were then given an additional supply of water and their signs diminished. On 25.7.55 P.1397 was killed.

On 26.7.55, the error in water supply was discovered and rectified, but none of the surviving pigs subsequently showed more than depression with some trembling and ataxia, and they were killed on 28.7.55 and 29.7.55.

The macroscopic appearance at necropsy was similar in all pigs, the only abnormalities being moistness and congestion of the brain, a few " milk spots " in the liver and about 100 c.c. of urine in the bladder.

Typical vascular lesions of meningo-encephalitis eosinophilica were present in the cerebra of 4 out of the 7 pigs, but none showed the regular mid-cortical band of malacia which is a characteristic but inconstant lesion of the disease.

This experiment is summarised in Table I.

Experiment II

Analytical grade sodium chloride was mixed with meal to give a final concentration of 9·4 per cent. by weight. Immediately before feeding, the meal was mixed with 2½ times its weight of water.

Four pigs were fasted on the evening of 8.9.55, and started on the experimental diet on 9.9.55. On the morning of 11.9.55, typical signs of meningo-encephalitis eosinophilica had appeared in 3 of the pigs, and 2 died that day. P.1636 appeared normal, but developed fine tremors on the next day.

By 14.9.55 P.1632 was *in extremis* and was killed. P.1636 continued to feed moderately, but showed tremors and seemed a little hyperaesthetic, and was killed on 15.9.55.

Histologically, typical vascular lesions of meningo-encephalitis eosinophilica were present to varying degrees in the cerebra of all 4 pigs, and the regular midcortical zone of malacia was demonstrable in the cerebrum of P.1634, the first pig to die.

The kidney of P.1632 showed considerable vacuolation of the epithelial cells of the convoluted tubules, which contained eosinophilic detritus and globules. Possibly these changes are indicative of some degree of tubular nephrosis, which may explain the low chloride content of this pig's urine.

This experiment is summarised in Table II.

In Experiments I and II, abnormalities of the central nervous system were detected only in the cerebrum.

Experiment III

In view of the above results and those of Smith using sodium proprionate the question arose whether the syndrome was due to direct toxicity of the sodium ion or to disturbances of water balance occasioned by the high osmotic tension of the diet. It was therefore decided to feed groups of pigs on diets containing urea alone, or urea with sodium chloride, at concentrations calculated to give an osmotic effect equivalent

TABLE I

EXPERIMENT I. EFFECTS OF FEEDING MEAL CONTAINING 5·3 PER CENT. NaCl

Number of pigs	Mode of death	Days to death	Salt eaten, gm. per kilo. bodyweight per day	White blood cell counts at time of death						Plasma chloride mg. per cent.	Lesions	
				Total	L per cent.	N per cent.	E per cent.	M per cent.	B per cent.		Perivascular eosinophile infiltration	Malacia
1397	K	5½	4·7	19,750	34·0	53	4·0	9	0	505	++++	—
1400	K	8½	3·3	12,200	65·0	27	1·0	7	0	480	—	—
1402	K	8½	1·8	24,300	63·0	25	6·0	6	0	575	+++	—
1403	K	8½	2·4	22,600	74·0	17	2·0	7	0	482	+	—
1398	K	9½	2·1	25,150	64·5	29	1·5	5	0	502	—	—
1399	K	9½	2·3	20,200	50·5	33	5·5	11	0	475	+	—
1401	K	9½	2·4	19,100	48·5	36	5·5	10	0	535	—	—

TABLE II

EXPERIMENT II. EFFECTS OF FEEDING MEAL CONTAINING 9·4 PER CENT. NaCl

Number of pig	Days to signs	Days to death	Mode of death	Salt eaten gm. per kilo. bodyweight per day	White blood cell counts at time of death						Urine chloride mg. per cent.	Serum chloride mg. per cent.	Lesions	
					Total	L per cent.	N per cent.	E per cent.	M per cent.	B per cent.			Perivascular eosinophile infiltration	Malacia
1634	2	2	D	4·8	Not done						Not done		+++	++
1631	2	2	D	4·3	"						615	Not done	+++	—
1632	2	5	K	4·9	4,050	12	79	2	7	0	142	425	+++	—
1636	4	6	K	3·7	15,600	40	50	2	8	0	1,133	473	++	—

to that of a concentration of about 7 per cent. sodium chloride.

In this experiment the calculated quantity of urea and/or sodium chloride was dissolved in the water which was mixed with the meal in a proportion of $2\frac{1}{2}$: 1 by weight.

Group A

Four pigs were placed on a diet of proprietary fattening meal (which contained 0·8 per cent. sodium chloride when purchased) and 15 per cent. urea.

The pigs were fasted on the evening of 2.10.56 and the experimental diet introduced on the morning of 3.10.56. On 4.10.56 all the animals seemed depressed and tended to shiver.

On the morning of 5.10.56, all were depressed and 3 had variable ataxia and tended to push their heads into the corners of the box. By the evening of the same day, the 3 affected pigs were recumbent whenever possible, and if roused, wandered aimlessly about, bumping into the walls and each other. P.4174 was depressed but only slightly ataxic.

On 6.10.56 P.4194 was recumbent and semi-comatose, P.4189 was recumbent with intermittent head-nodding, P.4174 and P.4180 were standing but markedly depressed and somewhat ataxic. P.4194 died at about midday following cardiac puncture.

On the morning of 7.10.56, P.4189 was found dead. P.4180 was semi-comatose with slight muscular tremors, and P.4174 was depressed with slight ataxia, amaurosis and constipation.

On 8.10.56 the condition of P.4180 was unchanged, and P. 4174 was also recumbent and semicomatose. Both pigs were killed about midday.

Necropsy revealed no consistent picture. The stomachs of all the pigs were well filled, and the livers showed various degrees of "milk spot." The alimentary contents of P.4194 and P.4189 were abnormally dry, and the arcuate vessels in the kidneys of P.4189 and P.4180 were congested. Urine volume varied from 150 ml. in P.4194 to 5 ml. in P.4174.

Histopathology. P.4194. Slight endothelial proliferation and perivascular lymphocytic infiltration affected the vessels of the cerebrum, and there were typical zones and foci of midcortical malacia.

P.4189. The vessels of the cerebrum had undergone moderate endothelial proliferation and lymphocytic infiltration, and traces of vacuolation were visible in the ground substance in the regions usually affected by malacia.

P.4174. A mild degree of endothelial proliferation and lymphocytic infiltration involved a number of cerebral vessels, and there were large zones of typical midcortical malacia.

P.4180. A number of cerebral vessels showed moderate perivascular infiltration and slight endothelial proliferation, and patches of midcortical malacia were widespread.

In all 4 pigs, the central nervous system other than the cerebrum showed no obvious abnormality, save for a moderate hyperaemia.

Group B

On the evening of 2.10.56, 4 pigs were fasted, and on the morning of 3.10.56 were introduced to a diet of the same meal (containing 0·8 per cent. sodium chloride) together with 7·5 per cent. urea and 3 per cent. additional sodium chloride. By the evening of 4.10.56, all the pigs seemed depressed and tended to shiver. On the morning of 5.10.56 P.4183 had typical signs of acute meningo-encephalitis, with head nodding, walking backwards and occasional convulsions, and died early in the afternoon. P.4188 and P.4193 showed typical less acute signs, amaurosis and aimless wandering, which became more severe later in the day when they pressed their heads against the walls and tripped over objects. P.4192 was merely depressed. On 6.10.56 P.4188 and P.4193 were recumbent with continual head nodding and occasional convulsions. P.4192 could walk but was very depressed, amaurotic and had occasional convulsions.

On 7.10.56, P.4188 was recumbent with continuous clonic spasms of the head and neck, P.4193 was semi-comatose with diffuse muscular spasms, P.4192 was recumbent with constant paddling movements of the limbs, staring eyes and occasional convulsions and was killed.

On 8.10.56, P.4188 was alive in the early morning but was found dead at noon.

P.4193 was recumbent and semicomatose, with constant clonic contractions of the muscles, and was killed.

Necropsy revealed well-filled stomachs in all pigs, with some hyperaemia of the mucous membrane. There was some fatty change in the liver of P.4183, and the arcuate vessels in the kidneys of P.4193 were congested.

Histopathology. P. 4183. Only minimal endothelial proliferation was detected in a few of the cerebral vessels, but numerous foci of malacia extended in a regular band round the midcortical zone of the cerebrum.

P.4192. Moderate endothelial proliferation and eosinophile infiltration was present in a number of cerebral vessels, and the ground substance of the cortex was considerably vacuolated in the region where malacia usually occurs.

P.4188. Large areas of malacia and vacuolation were visible in the cerebrum not only in the usual midcortical zone but also in the plexiform and outer granule layers. The vessels had developed only slight endothelial proliferation and lymphocytic infiltration with an occasional single eosinophile present in the vessel wall, but adjacent to a few of them were small areas of rather diffuse microgliosis.

P.4193. Many cerebral vessels showed considerable endothelial hyperplasia, sometimes accompanied by a little lymphocyte and eosinophile infiltration. A chain of foci of malacia ran around the midcortical zone, and areas of diffuse gliosis were scattered about the grey matter. In these 4 pigs, no abnormalities other than moderate hyperaemia was detected in the central nervous system outside the cerebrum.

Group C

In order to assess the effect of 3 per cent. added sodium chloride alone at this level of water intake, 3 pigs were subjected to a similar process to that

J. T. DONE, J. D. J. HARDING & M. K. LLOYD.—MENINGO-ENCEPHALITIS EOSINOPHILICA OF SWINE

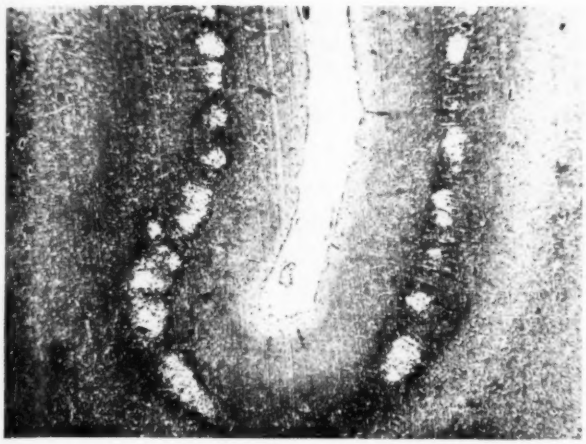

FIG. 1.—Pig. 4174 (15 per cent. urea). Cerebrum showing cortical laminar malacia. Masson's trichrome × 20 approx.

FIG. 2.—Pig 1634 (9·4 per cent. NaCl). Cerebrum showing cortical laminar malacia. Masson's trichrome × 20 approx.

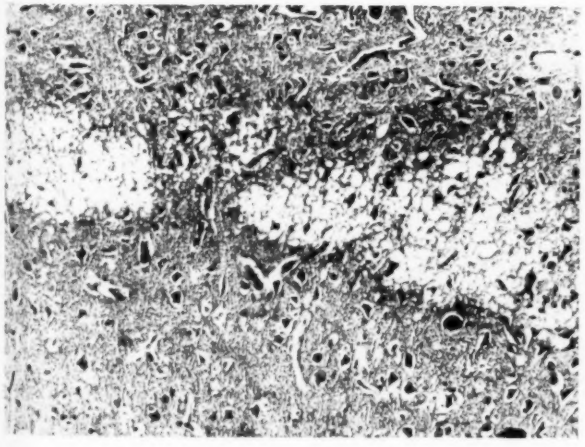

FIG. 3—Pig. 4174 (15 per cent. urea). Detail from Fig. 1, showing necrotic neurones and vacuolation of ground substance. Masson's trichrome × 90 approx.

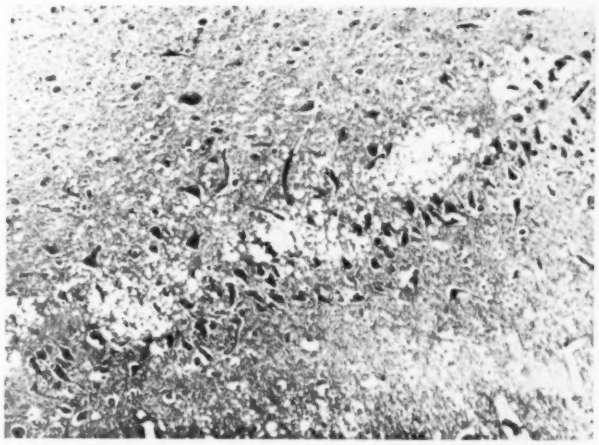

FIG. 4.—Pig 1634 (9·4 per cent. NaCl). Detail from Fig. 2, showing necrotic neurones and vacuolation of ground substance. Masson's trichrome × 90 approx.

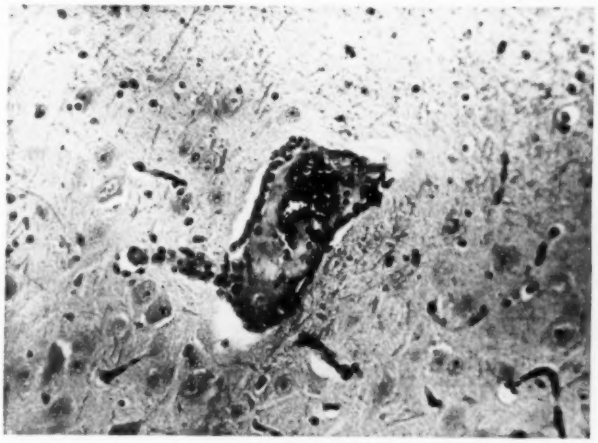

FIG. 5.—Pig 4174. (15 per cent. urea). Vessel in cerebral cortex showing mild perivascular lymphocytic infiltration and endothelial proliferation. H. & E. × 220 approx.

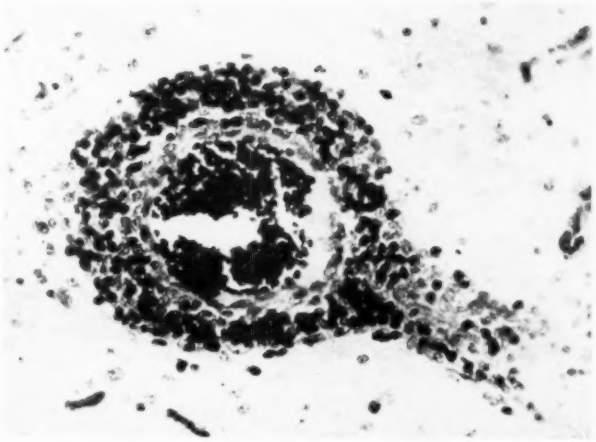

FIG. 6.—Pig 1634 (9·4 per cent. NaCl). Vessel in cerebral cortex showing severe perivascular eosinophile infiltration (dark cells) and endothelial proliferation. H. & E. × 220 approx.

TABLE III

EXPERIMENT III. EFFECTS OF FEEDING MEAL CONTAINING (A) 15 PER CENT. UREA; (B) 3 PER CENT. NaCl + 7·5 PER CENT UREA; (C) 3 PER CENT. NaCl

	Number of pigs	Days to signs	Days to death	Mode of death	Lesions					Serum chloride mg. per cent.	Remarks
					Perivascular eosinophile infiltration	Perivascular lymphocytic infiltration	Vascular endothelial proliferation	Microgliosis of cerebrum	Malacia of cerebrum		
A.	4194	2	3½	D/K	−	+	+	−	++	Not done	
15 per cent.	4189	2	4	D	−	+	+	−	±	,,	
urea	4174	3	5	K	−	+	+	−	+++	445	
	4180	2	5	K	−	+	±	−	++	500	
B.	4183	2	2	D	−	−	±	−	++	490	
3 per cent.	4192	3	4	K	++	−	++	−	±	500	Malacia irregularly distributed
NaCl	4188	2	5	D	−	−	+	+	+++	Not done	
7·5 per cent. urea	4193	2	5	K	+	+	++	++	++	490	
C.	4106	−	11	K	−	+	−	−	−	Not done	
3 per cent.	4107	−	11	K	−	±	−	−	−	,,	
NaCl	4108	−	11	K	−	−	−	−	−	,,	

D = Died. K = Killed. ± = Doubtful. − = Negative. + to +++ = ascending degrees of severity

used in A and B, save that only 3 per cent. of sodium chloride was added to the diet. The experiment was carried on for 11 days, in the course of which time the pigs became slightly dull and reluctant to eat and P.4106 occasionally showed slight ataxia and fine muscular tremors over the flanks. The pigs were killed on the morning of the 12th day.

Histopathology. P.4106 had slight perivascular round cell infiltration in a number of vessels of the cerebrum, with a little sponginess of the ground substance around a few of them. There was also a slight patchy non-suppurative leptomeningitis over the cerebellum.

P.4108 had traces of round cell infiltration in the cerebral leptomeninges and around a few vessels in the cerebral white matter and in the corpora quadrigemina.

P.4109 had slight, patchy round cell infiltration of the cerebellar leptomeninges and around one or two vessels in the corpora, where there was also one small focus of microgliosis.

None of these pigs demonstrated any of the typical lesions of meningo-encephalitis eosinophilica.

The results of Experiment III are summarised in Table III.

Discussion

Experiments I and II confirm that the classic lesions of meningo-encephalitis eosinophilica may be reproduced by feeding sodium chloride with restricted water, particularly when inadequate trough space encourages competitive eating. The wealth of literature recording difficulties in reproducing salt poisoning experimentally serves to emphasise the importance of water restriction in the pathogenesis of the disease and it is clearly meaningless to speak of a toxic dose of sodium chloride as if it were an absolute figure, without reference to the quantity of water available to the animal. At the same time it must be realised that the water supplied in these experiments (*i.e.* 2½ times the weight of dry meal) falls within the limits advocated as good pig husbandry (Watson & More, 1945 ; Braude, 1954).

Since sodium chloride, sodium proprionate (Smith, 1957) and sodium lactate (Hjärre & Öbel, 1956) have all been used successfully to reproduce meningo-encephalitis eosinophilica, it is often assumed that all the lesions of the disease are specific to sodium salt poisoning. Experiment III suggests that, while the perivascular eosinophile infiltration is so specific, mid-cortical laminar malacia and vascular endothelial proliferation, sometimes accompanied by a degree of perivascular lymphocytic infiltration and microgliosis, may be occasioned by other substances in high osmotic concentration in the diet ; indeed, that those lesions are probably more indicative of disturbed water balance in the individual than of any specific poisoning. In this respect the observations of Hjärre & Öbel (1956) and of Gellatly (1957) are of considerable interest. The former authors attempted to poison 6 pigs with ammonium chloride, and found in one a " lymphocytic encephalitis " of which they do not give details. Gellatly (1957) described a syndrome of depression, circling and opisthotonus in pigs under 2 months old, characterised pathologically by "laminar cerebral cortical necrosis," and usually associated with dehydration and anoxia. He also drew attention to a report by Douglas (1949) of rather similar lesions in man, occasioned by anoxia.

Hjärre & Öbel (1956) and Smith (1957) also noted that some of their experimental pigs died after sudden heavy water consumption, suggesting that a disturbance of water balance rather than a specific toxic agent was the operative factor.

Morrill (1957) was quoted as saying that lesions resembling those of meningo-encephalitis eosinophilica are seen in pigs poisoned with smartweed but, in a private communication (1958) he indicates that the statement was a verbal one made from memory and he does not care to state unequivocally that the lesions are identical.

It must, of course, be agreed that in field outbreaks

of meningo-encephalitis eosinophilica, as in experiments described by several authors, individual cases occur in which eosinophile infiltration is absent but some of the other typical lesions are invariably present. It would seem sound practice to make a firm diagnosis of sodium salt poisoning on neuropathological evidence alone when typical eosinophile infiltration is present ; in its absence the other lesions should only suggest a disturbed water balance.

Malacic foci with or without eosinophile infiltration, similar to those occurring in the cerebrum are sometimes seen in other parts of the brains from clinically similar cases of field disease (Hjärre & Öbel, 1956 ; Michalka, 1941) but no author has recorded lesions in experimental pigs elsewhere than in the cerebrum, and such extracerebral lesions should be interpreted with caution.

The precise mechanism by which the lesions arise remains obscure, but present observations suggest that dehydration plays a large part in the process. In man there is a maximal osmotic concentration of urine which varies with the rate of output, being higher for low minute volumes (Harvey, McCance & Taylor, 1946) so that 2 osmotically active substances cannot exist simultaneously in urine each at its maximum concentration. Furthermore, even in severe dehydration urine continues to be formed—the " urine obligatoire " of Ambard & Papin (1909). Assuming similar limits in pigs, the feeding of high osmotic concentrations of any solute which is absorbed and excreted, unchanged, by the kidney, could result in more water being required for their excretion than was available to the pig, causing the osmotic pressure of the body fluids to rise. Thus, in Experiment III, the feeding of urea occasioned disease in pigs receiving quantities of sodium chloride and water normally incapable of causing poisoning, the critical value being the total quantity of osmotically active substances being excreted by the kidney, relative to the quantity of water available from all sources.

Though limited in number the chloride values of the serum and plasma samples examined compare fairly closely with those of other workers, where only sodium chloride poisoning was involved. (Cox & Pitts (1957) found the average plasma chloride level from 3 normal pigs to be 354 mg. per cent., and the levels in 3 poisoned pigs to range from 441 to 486 mg. per cent. Smith (1957) found serum chloride levels in 14 pigs before being placed on experiment ranged from 350 to 408 mg. per cent., and levels in 10 pigs showing signs of sodium chloride poisoning ranged from 442 to 507 mg. per cent.) However, the results of Experiment III suggest that similar elevation of blood chloride may occur in the absence of high dietary concentrations of sodium salts, possibly due to the preferential excretion of urea preventing sodium chloride from reaching its normal osmotic concentration in the urine. The present work did not cover the estimation of sodium levels, which may be a more reliable diagnostic aid.

Smith (1957) noted an eosinopenia in the blood of his experimental pigs. Michalka (1942) mentions " a circulating eosinophilia " but gives no figures and may have intended to refer only to eosinophiles visible in the lumina of vessels seen in histological sections. The present authors found no obviously abnormal blood picture, but counts were performed only at the time of death and transient changes may have been missed.

Most diets fed to swine in this country probably contain adequate amounts of sodium and chloride (Done, 1957) and, in the light of our present knowledge of meningo-encephalitis eosinophilica and similar conditions, it seems wise that no sodium chloride should be added to the food, and that pigs should always have access to unlimited water supply.

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(*Part I was published in* THE VETERINARY RECORD *of January 26th, 1957, page 67.*)

THE ASSOCIATION OF VETERINARY STUDENTS' ANNUAL CONFERENCE

The XIth Annual Conference of the Association of Veterinary Students was opened in London by the Minister of Agriculture on Friday, January 23rd. There was a large attendance of veterinary students from the 7 veterinary colleges in the United Kingdom and Eire, and the occasion was also marked by the presence of some distinguished members of the profession, including Professor T. J. Bosworth (President of the Royal College of Veterinary Surgeons), Mr. H. F. Hebeler (President of the British Veterinary Association), and Mr. J. N. Ritchie (Chief Veterinary Officer of the Ministry of Agriculture). The Principal of the Royal Veterinary College, Dr. R. E. Glover, replied to the Minister's speech. A full report of the proceedings will be published in our next issue.

News and Comment

VETERINARY SURGEONS AND EXCHANGE VISITS TO THE U.S.A.

At a recent meeting of the Parliamentary and Public Relations Committee of the B.V.A. it was suggested that the forthcoming International Veterinary Congress in Madrid from May 21st to 27th would be bringing many American veterinary surgeons to Europe. It was felt that this might offer an excellent opportunity of extending the acquaintanceship between veterinary surgeons in this country and the United States, and if any member would like to offer a visiting American veterinary surgeon 2 or 3 weeks' hospitality he should communicate with the General Secretary of the B.V.A. at 7, Mansfield Street, Portland Place, London, W.1.

The Parliamentary and Public Relations Committee hope that these visits will enable veterinary surgeons in this country to see practice in America in exchange for similar facilities in the United Kingdom.

UNIVERSITY NEWS

Bristol

At a Degree Congregation held in the reception room of Bristol University on Monday, January 19th, the following were admitted to the Degree of Bachelor of Veterinary Science by the Vice-Chancellor, Sir Philip Morris:—

Richard Hereward Chapman; John Lawrence White Crittall; Thomas Hicks Roberts Davies; George David Jowett; Michael Geoffrey Prior; Bernard Anthony Kavanagh Swan; Michael Edwin Thomas Watts; Arthur John Westaway and Thomas Goodwin Yarrow.

During the same ceremony the degree of Doctor of Philosophy in the Faculty of Science was awarded to Mr. Raymond Robert Ashdown.

In a ceremony which followed the new graduates were admitted to membership of the Royal College of Veterinary Surgeons by Mr. J. B. White in the unavoidable absence of the President. In introducing Mr. White and Mr. Oates the Registrar, Professor Ottaway said how pleased they all were that, in the President's absence, Mr. White had been nominated to attend for, besides being a practising veterinary surgeon held in high esteem, he was a good friend of the Bristol Veterinary School.

After the graduates had taken the oath Mr. White spoke to the new members. He reminded them that they were entering a body whose standards were very high and urged them to uphold that standard. He expressed the wish that they would make every endeavour to maintain good relations with their neighbours, pointing out that this would be as much their responsibility as that of anyone else. The value of meetings of Divisions of the B.V.A. as a means of maintaining social contacts was brought to their notice in addition to the opportunity they brought for the interchange of knowledge. That such interchange was necessary Mr. White was sure for if his experience was any guide they would always be meeting new conditions.

After wishing them good health and happiness in their new work Mr. White congratulated Mr. Ashdown on his attainment of a higher degree.

GLASGOW UNIVERSITY VETERINARY MEDICAL ASSOCIATION

We are indebted to a correspondent for the following notes:—

The social side of G.U.V.M.A's. activities last term was given a fine start by the Freshers' Social which was held in October. As usual most of the latent talent in the college was unearthed and a most successful evening's entertainment was provided. Mr. S. Jennings, of course, provided some of his own inimitable humour to the evening.

The traditional field day with the "Dick" was held in November. Despite inclement weather, the 1st XV lost by only 3 points, and the 2nd XV drew at 3 points each. The soccer game was drawn 4-4, though the women students lost at hockey by 7 goals. The table tennis team was victorious and won by 2 games.

The Clinical Club held several successful meetings, and at one of them, which a party from the "Dick" attended, Dr. Holmes of the Bristol school gave a paper on metabolic diseases.

Two dates which graduates of the college may wish to note in their diaries are February 26th, the Annual Ball, and May 16th, the Annual Cruise, this year to Tignabruich.

FOWL PEST IN ESSEX

Since December 28th, 1958, 30 outbreaks of fowl pest have been confirmed in Essex. The county (with the exception of the county boroughs of East and West Ham) has therefore been declared an Infected Area by the Minister of Agriculture, Fisheries and Food under the Fowl Pest (Infected Areas Restrictions) Order, 1956.

The restrictions came into force on January 26th. Poultry may not be moved into, out of or within the Infected Areas except under licence. The holding of poultry store markets in the Area is prohibited.

EXPORTS OF DRUGS AND MEDICINES

During 1958 the export of British drugs and medicines to the U.S.A. reached the record figure of £1,100,000, compared with £830,000 in 1957. Exports to Egypt recovered, from £84,000 to £494,000, and the industry believes that the signing of the Anglo-Egyptian financial agreement will lead to considerably more sales in this market.

Figures published by the Board of Trade show that increased pharmaceutical exports went to Nigeria, New Zealand, the Irish Republic, Pakistan, U.S.A. and Canada. There was a slight fall in exports to some countries; the financial crisis and stringent import policy in India for instance resulted in a decrease in exports to that country from £2.9 million to £1.7 million. Total exports (£37.8 million) were second only to the record figure (£39.6 million) reached in 1957.

Antibiotics made up the biggest section of this trade (£8,300,000) with vitamins next (£2,500,000). Exports of alkaloids, aspirin and sulphonamide preparations each exceeded £1,000,000.

NEW APPOINTMENTS

The Prime Minister has appointed Sir Alan Hitchman, K.C.B., Permanent Secretary of the Ministry of Agriculture, Fisheries and Food, to succeed Mr. W. Strath, C.B., as a full-time Member of the United Kingdom Atomic Energy Authority. Sir Alan Hitchman will have special responsibility for the external relations and commercial policy of the Authority. His appointment will take effect from April 1st, 1959.

The Minister of Agriculture, Fisheries and Food, with the approval of the Prime Minister, has appointed Mr. A. J. D. Winnifrith, C.B., a Third Secretary in the Treasury, to succeed Sir Alan Hitchman as Permanent Secretary of the Ministry.

PERSONAL

Marriage

HAMILTON—O'KANE.—On December 29th, 1958, at St. John's Church, Port Glasgow, Renfrewshire, Arthur F. Hamilton, B.V.M.S., M.R.C.V.S., of 1 Hillfoot Avenue, Bearsden, Glasgow, to Kathleen O'Kane of Woodbank, Port Glasgow.

Dr. R. R. Ashdown received his degree of Ph.D., at a Congregation of the University of Bristol held on January 19th. The subject of Dr. Ashdown's thesis was "Observations on the Penis and Sheath of the Castrated Ox."

COMING EVENTS

February

4th (Wed.). Meeting of the West of Scotland Division at the Veterinary Hospital, Bearsden Road, Bearsden, Glasgow, 2.30 p.m.

5th (Thurs.). Meeting of the Central Veterinary Society at the Royal Veterinary College, Camden Town, N.W.1, 6 p.m.

6th (Fri.). Meeting of the Shropshire Veterinary Club at the Oak Hotel, Shelton, Shrewsbury, 8 p.m.

11th (Wed.). Annual General Meeting of the Society for the Study of Animal Breeding at the Royal Society of Medicine, 1, Wimpole Street, W.1.

7th Annual General Meeting of the Society for the Study of Animal Breeding, in the West Hall, R.S.M., 1, Wimpole Street, London, W.1, 11.30 a.m.

13th (Fri.). General Meeting of the Midland Counties Veterinary Association at the Angel Hotel, Ludlow, Salop., 2.30 p.m.

19th (Thurs.). Meeting of the B.S.A.V.A. Midland Area at the Chace Hotel, Coventry, 7.30 p.m.

Meeting of the Essex Veterinary Society at the Essex Institute of Agriculture, Writtle, Essex, 7.30 p.m.

26th (Thurs.). Annual Ball of Glasgow University Veterinary Association at the Càdoro Restaurant.

27th (Fri.). Annual Ball of the Liverpool University Veterinary Society in the Students Union.

March

10th (Tues.). Annual General Meeting of the Eastern Counties Veterinary Society at the Bell Hotel, Norwich.

20th, 21st and 22nd (Fri., Sat. and Sun.). Conference of the British Small Animal Veterinary Association in London.

April

8th, 9th and 10th (Wed., Thurs., and Fri.). B.V.A. Committee and Council Meetings in Edinburgh.

August

30th to 5th September. 77th Annual Congress of the British Veterinary Association in Folkestone. **(Official opening Monday, August 31st.)**

ADDRESSES OF DISEASE INFECTED PREMISES

The list given below indicates, first the county in which are situated the premises on which disease has been confirmed, followed by the postal address and date of outbreak.

Anthrax

Lanarks. Laigh Cleuchearn Farm, East Kilbride, Glasgow (Jan. 22).
Norfolk. Larkeys Farm, East Ruston, Norwich (Jan. 26).
Worcs. Stud Farm, Stockwood, Redditch (Jan. 20).

Fowl Pest

Dorset. 6 Lawn Farm, Gillingham (Jan. 20).
Essex. Ldderstyle Farm, Epping; Stapleford Hall Cottage, Stapleford Abbott, Romford; Millfields General Stores, Rayne, Braintree; White Gates, Boreham, Chelmsford (Jan. 20); Foxborough, Hedingham Road, Halstead; Typonds, Rayne, Braintree; Knights Farm, Colne Engaine, Halstead (Jan. 21); Hill Farm, Woodham Ferrers, Chelmsford; Lakes Farm, Rayne, Braintree; Kingstones Farm, White Roothing, Dunmow (Jan. 22); The Falcon Castle, Hedingham, Halstead; Chaplins Poultry Yard, Beehive Lane, Galleywood, Chelmsford; Brook Street House, Halstead (Jan. 23); 2 Church Road, Boreham, Chelmsford; Sunningdale, Braintree Green, Rayne (Jan. 24); Springette, Queensborough Lane, Rayne (Jan. 25); Fairstead Lodge, Terling, Chelmsford (Jan. 26).
Kent. Quorum, Ashford Road, New Inn Green, Hythe (Jan. 21); Bilham Farm, Marsham, Ashford (Jan. 23).
Norfolk. Grange Farm, Lessingham (Jan. 20).
Suffolk. Church Farm, Chediston, Halesworth (Jan. 21).
Wilts. Blackhouse Farm, Mere, Warminster (Jan. 23).
Yorks. Devonshire House, Sinderby, Thirsk (Jan. 24).

Swine Fever

Derbys. Lower House Farm, Sheen, Buxton (Jan. 20).
Essex. Crouchmans Farm, Ulting (Jan. 22); Buttsbury Lodge, Stock, Ingatestone (Jan. 26).
Glam. Hillside, Radyr Court Road, Llandaff, Cardiff, (Jan. 26).
Hants. Lower Posbrook Farm, Meon, Titchfield, Fareham (Jan. 21).
Hunts. Station Pig Farm, R.A.F. Station, Wyton; Wistow Toll, Wistow (Jan. 21).
Lancs. Rossbottom Farm, Northwich Corner, Aston, Preston Brook, Warrington (Jan. 26).
Norfolk West View, Browston, Great Yarmouth (Jan. 22).
Notts. Paylings Piggeries, Granby, Nottingham (Jan. 20).
Suffolk. 41 Jackson Road, Newbourne, Woodbridge (Jan. 21); Boydland Farm, Capel St. Mary, Ipswich (Jan. 22).
Warwicks. Alma Croft Farm, High Street, Solihull Lodge, Shirley, Solihull (Jan. 21).
Wilts. Blackland Farm, Blackland, Calne (Jan. 20).
Yorks. Holme Dene, Muston, Filey (Jan. 23).

Letters to the Editor

The views expressed in letters addressed to the Editor represent the personal opinions of the writer only and their publication does not imply endorsement by the B.V.A.

SCIENTIFIC

Preliminary Note on the Propagation of Canine Distemper Virus in Different Tissue-Culture Systems

Sir,—It has recently been reported by Rockborn (1958) that the virus of canine distemper is propagable in cultures of canine kidney with the production of cytopathogenic effects and multinucleated cells. In general, the findings of the Swedish worker are in accord with those observed in this laboratory during the past year. Several additional facts have accrued from our work, however, which seem worthy of record at the present time.

(a) *Methods of isolation.* The virus has been successfully propagated in several tissue-culture systems the first of which was procured from the kidneys of ferrets that had been inoculated experimentally with material from clinically affected dogs. Similar results were subsequently obtained with cultures of pulmonary and renal tissues taken from naturally infected dogs. Later still, the virus was found to grow in cultures of healthy canine lung and kidney and in healthy ferret kidney monolayers after they had been inoculated with the blood or with suspensions of organs secured from infected dogs and ferrets. Finally, the virus has been found to respond to serial passage in tissue-cultures and further to be successfully passaged from cultures of canine origin to cultures of ferret origin and *vice versa*. So far, virus has been propagated and isolated from one or both organs of 12 naturally infected dogs, from the kidneys of 7 experimentally infected ferrets and from numerous cases of distemper-affected dogs by inoculating material from infected animals to healthy tissue cultures, with subsequent passages.

To establish tissue-cultures small pieces of renal cortex or of lung were treated by trypsin 1:250 (Difco certified) diluted to 0.25 per cent. (w/v) with phosphate buffered solution (Dulbecco & Vogt, 1954; Youngner, 1954) or by overnight trypsinisation in basic Hank's solution (Bodian, 1956). Six hundred thousand to 1,000,000 cells per tube were seeded in either 199 synthetic mixture (Glaxo) or Hank's medium with 0.5 per cent. lactalbumin hydrolysate. Both media were supplemented by the addition of filtered and inactivated horse serum (Burroughs Wellcome No. 2) to a concentration of 10 per cent. in the case of the former and 20 per cent. in respect of the latter.

Sources of virus comprised (a) the supernatant fluid after centrifugation of a 20 per cent. suspension of infected tissues in the selected medium; (b) macerated blood clot and (c) medium from tissue cultures that manifested clear signs of cytopathogenicity.

(b) *Cytopathogenic effects.* Since the cytopathogenic effects were not easily discernible in living cultures under the microscope, on seeding the cells in each tube a cover slip was inserted and on this cover slip part of the monolayer was formed. From each batch of cultures a tube was withdrawn at intervals of 2 to 3 days and the contained cover slip was removed, washed in normal saline, fixed in corrosive sublimate-formol and stained by the haemulum-phloxin-tartrazine method after mordaunting in potassium dichromate. In this way cellular changes and cytopathogenicity were studied serially, particular attention being paid to the formation of inclusion bodies. All positive cultures have been characterised by a similar cytopathogenic effect, of which the most outstanding feature was the development of multinucleated cells containing from a few to scores of nuclei together with acidophilic inclusion bodies of mainly cytoplasmic location. The nuclei of the multinucleated cells were very often arranged in rings (Fig. 2), outside which large masses of phloxinophilic material could be seen. Apart from the formation of multinucleated cells individual cells, both epithelial and fibroblastic, manifested vacuolation, degeneration and the formation of intracytoplasmic inclusion bodies. The inclusions were brilliantly phloxinophilic, pleomorphic and resembled those to be observed in the tissues of animals suffering from distemper (Fig. 3). They stained much more intensely than the larger masses of phloxinophilic material, but even the latter occasionally stained equally brilliantly. Dull purple intranuclear inclusion bodies have been also observed particularly in cultures kept for over three weeks after inoculation.

The cytopathogenic picture closely resembles that of tissue-cultures infected with the virus of measles (Ruckle, 1957), a similarity to be laid alongside the serological evidence (Adams & Imagawa, 1957) which already suggests a relationship between the 2 viruses.

Cytopathogenicity was observed as early as 6 to 7 days in direct cultures of organs from an infected animal, whereas the time of appearance of cytopathogenicity in healthy cultures inoculated with suspension of virus varied considerably. Thus, cultures inoculated with blood or organ suspensions from an early acute distemper manifested cytopathogenicity as early as 3 to 4 days whereas 4 to 5 weeks were required in the case of material taken from advanced cases of canine distemper. This variation is apparently due to the different amounts of inoculated virus. Since blood, taken at an early stage of distemper infection, produces cytopathogenicity in a few days, work is in progress with a view to establishing a laboratory method of diagnosing distemper.

Studies on the relative susceptibility of healthy monolayers from ferret kidney, dog kidney and dog lung showed that of the 3 tested tissue-culture systems the most susceptible is ferret kidney and of

the 2 canine cultures, lung is slightly more susceptible than kidney. Thus the choice for primary isolation of virus is monolayers of renal ferret tissues. Susceptibility is being further investigated in order to find a more convenient tissue-culture system.

Identification of the isolated virus has been carried out by infecting ferrets with culture material and also by means of neutralisation tests in tissue cultures using a hyperimmune canine serum (kindly supplied by the Wellcome Research Laboratories) and convalescent canine sera.

Fuller details of the present work will be published later.

Yours faithfully,
J. T. VANTSIS.

University of Glasgow,
The Veterinary School,
83, Buccleuch Street,
Glasgow, C.3.

January 21st, 1959.

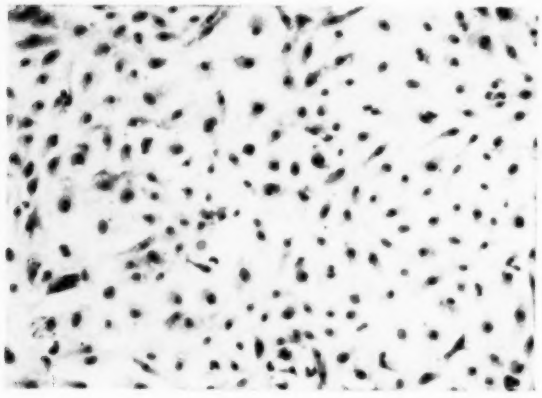

FIG. 1.—19-day-old culture. Normal ferret kidney. ×65.

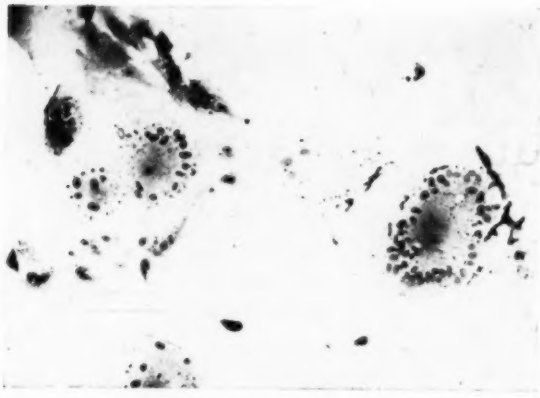

FIG. 2.—16-day-old culture; ferret kidney. Inoculated 8 days previously with blood from an infected ferret. Large cells with multiple nuclei in rings. Numerous cytoplasmic inclusion bodies. ×65.

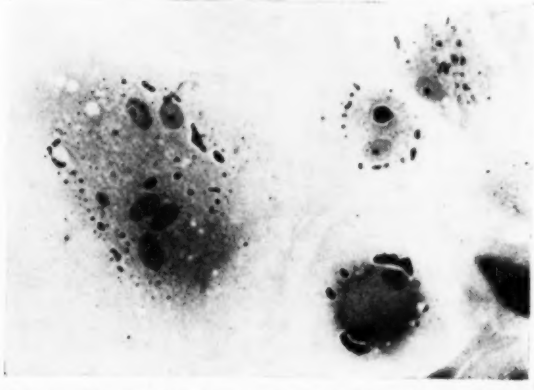

FIG. 3.—27-day-old culture; canine kidney from a clinical case of distemper. Multinucleated cells with abundant inclusions. ×145.

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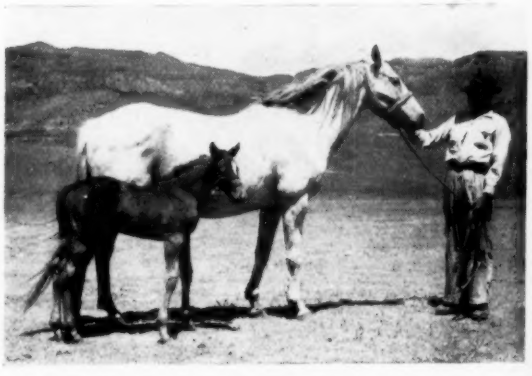
GENERAL

Caesarean Operation on a Mare

Sir,—In November, 1957, I performed a caesarean operation of the Thoroughbred mare, "Smart Girl," pictured below, and a report of this operation was published in THE VETERINARY RECORD of July 5th, 1958. This mare gave birth to a healthy foal without any assistance in November, 1958—just 1 year and 3 weeks after the operation.



The accompanying photograph has been sent to prove to the sceptics that this mare foaled again and without any difficulty. The incision was made in the right flank and readers will notice that the scar is no longer visible.

Yours faithfully,
M. SAS KROPIWNICKI.

Box 63,
c/o Graham and Brownlee Streets,
Barkly East, C.P.
Union of South Africa.

January 22nd, 1959.